

thymidine-3'-amidite (RIC, Inc.), 2'-O-MOE-G-3'-amidite (RI Chemical), 2'-O-methoxyethyl-5-methylcytidine-3'-amidite, 2'-O-methoxyethyl-adenosine-3'-amidite (RI Chemical), and 5-methylcytidine-3'-amidite. 3'-propylphthalimido-A and 2'-propylphthalimido-A were used as the LCA-CPG solid support. The required amounts of the amidites were placed in dried vials, dissolved in acetonitrile (unmodified nucleosides were made into 1M solutions and modified nucleosides were 100 mg/mL), and connected to the appropriate ports on a Millipore Expedite™ Nucleic Acid Synthesis System. Solid support resin (60 mg) was used in each column for 2X1 μmole scale synthesis (2 columns for each oligo were used). The synthesis was run using the IBP-PS(1 μmole) coupling protocol for phosphorothioate backbones and CSO-8 for phosphodiester. The trityl reports indicated normal coupling results.

[0197] After synthesis the oligonucleotides were deprotected with conc. ammonium hydroxide(aq) containing 10% of a solution of 40% methylamine (aq) at 55 °C for approximately 16 hrs. Then they were evaporated, using a Savant AS160 Automatic SpeedVac, (to remove ammonia) and filtered to remove the CPG-resin. The crude samples were analyzed by MS, HPLC, and CE. Then they were purified on a Waters 600E HPLC system with a 991 detector using a Waters C4 Prep. scale column (Alice C4 Prep.) and the following solvents: A: 50 mM TEA-Ac, pH 7.0 and B: acetonitrile utilizing the AMPREP2@ method. After purification the oligonucleotides were evaporated to dryness and then detritylated with 80% acetic acid at room temp. for approximately 30 min. Then they were evaporated.

The oligonucleotides were dissolved in conc. ammonium hydroxide and run through a column containing Sephadex G-25 using water as the solvent and a Pharmacia LKB SuperFrac fraction collector. The resulting purified oligonucleotides were evaporated and analyzed by MS, CE, and HPLC.

Table VI

SEQ ID NO. #	Oligonucleotides bearing Aminopropyl Substituents		Backbone
	(ISIS)#	Sequence (5'-3') <sup>1</sup>	
19	(23185-1)	A* <u>TG-CAT-TCT-GCC-CCC-AAG-GA</u> *	P=S
19	(23186-1)	A* <u>TG-CAT-TCT-GCC-CCC-AAG-GA</u> *	P=S
20	(23187-1)	A* <u>T<sub>0</sub>G<sub>0</sub>-C<sub>0</sub>A<sub>0</sub>T<sub>0</sub>-T<sub>0</sub>C<sub>0</sub>T<sub>0</sub>-G<sub>0</sub>C<sub>0</sub>C<sub>0</sub>-C<sub>0</sub>C<sub>0</sub>-A<sub>0</sub>A<sub>0</sub>G<sub>0</sub>-G<sub>0</sub>A*</u>	P=S/P=O
20	(23980-1)	A* <u>T<sub>0</sub>G<sub>0</sub>-C<sub>0</sub>A<sub>0</sub>T<sub>0</sub>-T<sub>0</sub>C<sub>0</sub>T<sub>0</sub>-G<sub>0</sub>C<sub>0</sub>C<sub>0</sub>-C<sub>0</sub>C<sub>0</sub>-A<sub>0</sub>A<sub>0</sub>G<sub>0</sub>-G<sub>0</sub>A*</u>	P=S/P=O
19	(23981-1)	A* <u>TG-CAT-TCT-GCC-CCC-AAG-GA</u> *	P=S
19	(23982-1)	A* <u>TG-CAT-TCT-GCC-CCC-AAG-GA</u> *	P=S

[0198] <sup>1</sup>All underlined nucleosides bear a 2'-O-methoxyethyl substituent; internucleotide linkages in PS/PO oligonucleotides are indicated by subscript >s= and >o= notations respectively; A\* = 3'-aminopropyl-A; A\* = 2'-aminopropyl-A; C = 5-methyl-C

Table VII

## Aminopropyl Modified Oligonucleotides

ISIS #	Expected Mass	Observed	HPLC	CE	Crude Yield (Ods)	Final Yield (Ods)
	(g/mol)	Mass Retention (g/mol)	Retention Time(min)	Retention Time		
23185-1	6612.065	6610.5	23.19	5.98	948	478
23186-1	7204.697	7203.1	24.99	6.18	1075	379
23187-1	7076.697	7072.33	23.36	7.56	838	546
23980-1	7076.697	7102.31	23.42	7.16	984	373
23981-1	7204.697	7230.14	25.36	7.18	1170	526
23982-1	6612.065	6635.71	23.47	7.31	1083	463

**EXAMPLE 56*****In vivo* stability of modified oligonucleotides**

[0199] The *in vivo* stability of selected modified oligonucleotides synthesized in Examples 49 and 55 was determined in BALB/c mice. Following a single i.v. administration of 5 mg/kg of oligonucleotide, blood samples were drawn at various time intervals and analyzed by CGE. For each oligonucleotide tested, 9 male BALB/c mice (Charles River, Wilmington, MA) weighing about 25 g were used. Following a one week acclimatization the mice received a single tail-vein injection of oligonucleotide (5 mg/kg) administered in phosphate buffered saline (PBS), pH 7.0. One retro-orbital bleed (either at 0.25, 0.5, 2 or 4 h post-dose) and a terminal bleed (either 1, 3, 8, or 24 h post-dose) were collected from each group. The terminal bleed (approximately 0.6-0.8 mL) was collected by cardiac puncture following ketamine/xylazine anesthesia. The blood was transferred to an EDTA-coated collection tube and centrifuged to obtain plasma. At termination, the liver and kidneys were collected from each mouse. Plasma and tissue homogenates were used for analysis to determine intact oligonucleotide content by CGE. All samples were immediately frozen on dry ice after collection and stored at -80C until analysis.

[0200] The CGE analysis indicated the relative nuclease resistance of 2',5'-linked oligomers compared to ISIS 11061 (Table III, Example 51) (uniformly 2'-deoxy-phosphorothioate oligonucleotide targeted to mouse *c-raf*). Because of the nuclease resistance of the 2',5'-linkage, coupled with the fact that 3'-methoxyethoxy substituents are present and afford further nuclease protection the oligonucleotides ISIS 17176, ISIS 17177, ISIS 17178, ISIS 17180, ISIS 17181 and ISIS 21415 were found to be more stable in plasma, while ISIS 11061 (Table III) was not. Similar observations were noted in kidney and liver tissue. This implies that 2',5'-linkages with 3'-methoxyethoxy substituents offer excellent nuclease resistance in plasma, kidney and liver against 5'-exonucleases and 3'-exonucleases. Thus oligonucleotides with longer durations of action can be designed by incorporating both the 2',5'-linkage and 3'-methoxyethoxy motifs into